

Allergens, IgE, mediators, inflammatory mechanisms

Dose-dependent effects of capsaicin nasal challenge: In vivo evidence of human airway neurogenic inflammation

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Background: Nerve involvement has been implicated in the pathophysiology of chronic respiratory inflammatory diseases. Peptidergic nerve stimulation has been shown to induce leukocyte activation and plasma extravasation in the airways of various animal species. The occurrence of this phenomenon of neurogenic inflammation in the human airway, however, has not been established.

Objective: We conducted this study to determine whether neuronal stimulation can induce reproducible and dose-dependent inflammatory changes in the human upper airway. **Methods:** Ten volunteers with active allergic rhinitis participated in the study. Capsaicin, the pungent component of hot pepper that specifically stimulates afferent nerve fibers, was administered by means of nasal spray in doses of 1 µg, 10 µg, and 100 µg in a double-blind, randomized, crossover manner with 1 week between doses. Symptom scores before and after capsaicin nasal challenge were recorded by using visual analog scales. Nasal lavage fluids collected before and at 30 minutes, 1 hour, and 4 hours after capsaicin challenge were analyzed for leukocyte counts; albumin and lysozyme levels were measured to evaluate effects on plasma leakage and gland secretion, respectively.

Results: Capsaicin nasal challenge produced symptoms of burning, congestion, and rhinorrhea. Leukocyte counts or albumin and lysozyme levels were not significantly increased after administration of 1 µg of capsaicin at any time point. On the other hand, there were significant increases in leukocyte counts 1 hour ($p < 0.05$) and 4 hours ($p = 0.008$) after 10 µg of capsaicin and 30 minutes ($p = 0.009$), 1 hour ($p = 0.007$), and 4 hours ($p = 0.007$) after 100 µg of capsaicin. Albumin and lysozyme levels were both significantly increased 30 minutes after 10 µg and 100 µg of capsaicin ($p = 0.005$ for both). Comparison of changes in symptom scores, leukocyte counts, and albumin and lysozyme levels among the three capsaicin challenges indicated generally increasing effects with higher capsaicin doses.

Conclusion: Capsaicin-sensitive nerve stimulation in subjects with active allergic rhinitis produces reproducible and dose-dependent leukocyte influx, albumin leakage, and glandular secretion. These results provide in vivo evidence for the occurrence of neurogenic inflammation in the human upper airway with active allergic disease. (*J Allergy Clin Immunol* 1997;100:632-41.)

Key words: Neurogenic inflammation, capsaicin, nasal lavage, leukocytes, albumin, lysozyme

In 1967 Jancso et al.¹ reported that cutaneous application of irritants in rodents produces an inflammatory response, which they termed *neurogenic inflammation*. Similar observations have since been ascribed to neuropeptides, such as tachykinins, released antidromically from sensory nerve endings on activation.² Recent studies have demonstrated that such neurally mediated inflammation occurs in the airways of various animal species. In the rat trachea, for example, nerve stimulation has been shown to induce leukocyte activation^{3,4} and plasma extravasation.⁵ In rat⁶ and guinea pig⁷ nasal mucosae, neuronal stimulation produces increased vascular permeability. In addition to these classical markers of inflammation, neuropeptide-mediated airway smooth muscle contraction has likewise been demonstrated in rabbits⁸ and ferrets.⁹ Further, sensory nerve stimulation in guinea pig¹⁰ and ferret¹¹ nasal mucosae has been shown to induce glandular hypersecretion.

Interestingly, most of these neurally mediated changes are similar to the typical features seen in chronic airway inflammatory diseases such as asthma¹² and rhinitis.¹³ On this basis, neuronal involvement in the pathophysiology of these respiratory conditions has been suggested.¹⁴ A major argument against this proposal, however, has been the paucity of evidence for the occurrence of this phenomenon in the human respiratory system.¹⁵

The human airway inflammatory response can be assessed by using the nasal challenge model, which provides convenient access for the application of appropriate agents and permits repeated collection of samples for evaluation.^{16,17} A pharmacologic agent that has proved useful in the investigation of effects of neuronal stimulation is capsaicin, the pungent component of hot pepper. Capsaicin specifically stimulates afferent nerves consisting mostly of unmyelinated C fibers and some

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Abbreviations used

ANOVA: Analysis of variance
HSA: Human serum albumin

myelinated A delta fibers.¹⁸ As a result, it can trigger central and axonal reflexes, the latter being putatively mediated by the release of tachykinins and other neuropeptides. Our group and others have shown that capsaicin nasal challenge in human beings induces symptoms of burning, lacrimation, and rhinorrhea, as well as increases in the lactoferrin and total protein content of nasal secretions.¹⁹⁻²¹ In a recent study we demonstrated that capsaicin, compared with its vehicle control, induces significant increases in the leukocyte content of nasal lavage fluids collected at various time points up to 4 hours after nasal challenge.²² Similar findings have been reported by Roche et al.,²³ although their observations were limited to early time points.²³ We have also found that nasal administration of capsaicin in subjects with active allergic rhinitis produces modest but significant increases in albumin levels of nasal lavage fluids.²⁴ This study was designed to further characterize these observations by determining whether the inflammatory cell influx and albumin leakage after sensory nerve stimulation are dose-dependent. Because these observed capsaicin-induced inflammatory effects were relatively modest, compared with those seen after antigen challenge, the demonstration of dose-responsiveness would further substantiate this phenomenon. In addition, the secretory response to capsaicin nasal challenge was likewise assessed by monitoring levels of the serous glandular product, lysozyme.

METHODS

Subjects

Ten volunteers (6 men and 4 women, ages 28 to 68 years) who had active allergic rhinitis participated in the study. All subjects had sensitivity to at least two aeroallergens, confirmed by skin testing. They gave a history of perennial nasal symptoms that were generally worse during the period from August to September when the study was conducted. The subjects avoided the use of antihistamines or α -adrenergic agonists for at least 1 week and steroids or cromolyn for at least 1 month before study entry. All participants gave informed consent, and the study was approved by the Institutional Review Board of the Johns Hopkins Bayview Medical Center.

Capsaicin spray challenge

Three dosages of capsaicin (Sigma Chemical Co., St. Louis, Mo.)—1 μ g, 10 μ g, and 100 μ g—were administered separately once a week in a double-blind, randomized, crossover manner. Dosages were delivered as 20 μ mol/L, 200 μ mol/L, and 2 mmol/L solutions, respectively, each dissolved in 5% ethyl alcohol and 5% Tween-80 (Sigma Chemical Co.). All challenges were delivered into both nostrils by using a metered nasal spray that delivered 75 μ l per actuation (coefficient of variation = 10%).

Nasal lavage

Nasal secretions were collected according to previously described methods.²⁵ Lavages were performed with lactated Ringer's solution (Kendall McGaw Laboratories Inc., Irvine, Calif.) prewarmed to 37° C and instilled into both nostrils with a pipette while the head was extended, then expelled into a plastic collecting basin after about 10 seconds. These samples were then transferred into conical tubes and stored on ice before further processing. At every time point when nasal fluid sampling was to be done, two nasal lavages with 5 ml then 10 ml of lactated Ringer's solution were performed successively to optimize collection of leukocytes.

Cytologic analysis

The nasal lavage samples were centrifuged at 2500 g for 15 minutes at 4° C. The supernatant of the 5 ml lavage sample was stored in aliquots at -20° C for later assays. The cell pellets from each pair of 5 ml and 10 ml lavages were combined and resuspended in 1 ml of lactated Ringer's solution. A 10 μ l aliquot of the cell suspension was then placed on a hemocytometer (American Optical, Buffalo, N.Y.), and the total leukocyte count was determined. On the basis of this cell count, the sample was diluted with lactated Ringer's solution to a maximal concentration of 5×10^5 leukocytes/ml. Microscopic slides were prepared by cytocentrifuging a 200 μ l cell suspension at 600 rpm for 6 minutes (Cytospin; Shandon Southern Instruments, Sewickley, Pa.). The slides were stained with a modified Wright stain (Diff-Quik; Baxter Healthcare Corp., McGaw Park, Ill.). Differential counts were then performed by a single reader who counted 200 leukocytes and categorized them as neutrophils, eosinophils, or mononuclear cells.

Albumin assay

Human serum albumin (HSA) levels in the lavage samples were determined by using an ELISA as previously described.²⁶ A 96-well ELISA plate was coated with a 1:900 dilution of sheep anti-HSA antibody (The Binding Site Ltd., Birmingham, England) and incubated overnight at 4° C. After washing the wells with buffer, nonspecific binding sites were blocked with 1% sheep serum incubated for 30 minutes at 37° C. The wells were then washed, and appropriate dilutions of the samples and HSA standard (Sigma Chemical Co.) were placed and incubated for 90 minutes at 37° C. After washing, a 1:1200 dilution of horseradish peroxidase-conjugated sheep anti-HSA (Dako Corp., Carpinteria, Calif.) was added and incubated for another 90 minutes at 37° C. The wells were then washed, the reaction was developed with o-phenylenediamine dihydrochloride (Sigma Chemical Co., St. Louis, Mo.), and the absorbance was read at 490 nm. The assay standard curve for HSA ranged from 1 to 256 ng/ml.

Lysozyme assay

Lysozyme levels in the lavage samples were determined with ELISA as described above by using a 1:400 dilution of sheep anti-human lysozyme antibody (Dako Corp.) as the primary antibody and a 1:1500 dilution of horseradish peroxidase-conjugated sheep anti-human lysozyme (The Binding Site Ltd.) as the secondary antibody. The assay curve for the lysozyme standard (Calbiochem, La Jolla, Calif.) ranged from 0.5 to 64 ng/ml.

Symptom scores

Subjects recorded symptoms of burning, congestion, and rhinorrhea using 10 cm long visual analog scales, which we have

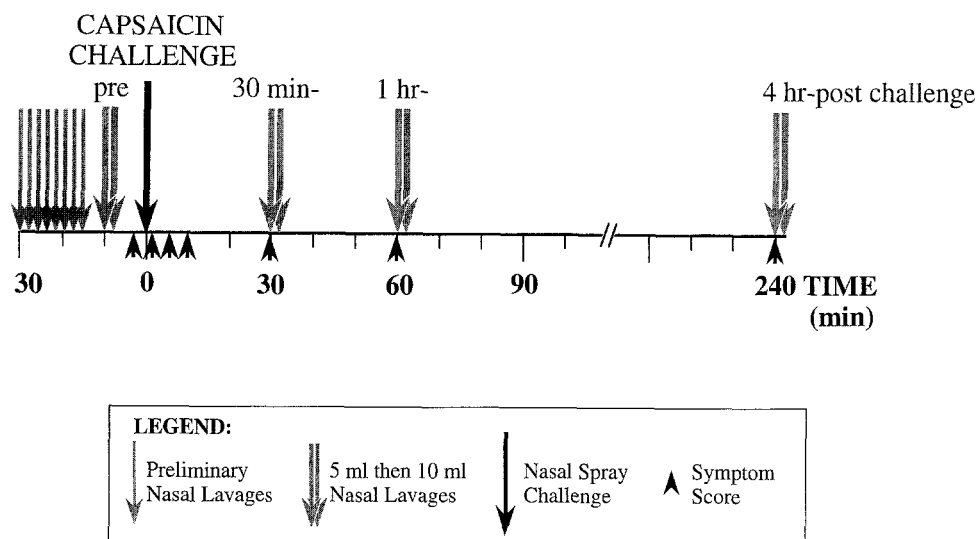


FIG. 1. Design of study. Subjects underwent nasal spray challenges with 1 μ g, 10 μ g, and 100 μ g of capsaicin, given 1 week apart in a double-blind, randomized, crossover manner. Nasal lavage samples collected before and after challenge were analyzed for leukocyte counts, as well as for albumin and lysozyme levels.

applied in several previous studies.^{19, 22, 24} This system yielded a continuous score from 0 to 10, ranging from "no sensation that you can notice" to "worst sensation that you could imagine."

Protocol

To wash out preexisting cells and other biologic components in the nasal secretions before capsaicin administration, eight nasal lavages were performed at the beginning of the study period, and the returned fluids were discarded. Pairs of successive lavages with 5 ml then 10 ml of lactated Ringer's solution were thereafter performed before challenge, then at 30 minutes, 1 hour, and 4 hours after capsaicin challenge (Fig. 1). The leukocyte, albumin, and lysozyme content of nasal lavage fluids collected at these time points was determined. Symptom scores were obtained before challenge and then at 10 seconds, 5 minutes, 10 minutes, 30 minutes, 1 hour, and 4 hours after capsaicin administration.

Data analysis

Nonparametric statistics were applied. To evaluate the effects of capsaicin administration, values before and after capsaicin challenge were compared by using Friedman's analysis of variance (ANOVA), followed by the Wilcoxon signed-rank paired test (with StatView 4.5 software; Abacus Concepts Inc., Berkeley, Calif.). Data are presented as medians of changes (Δ) from prechallenge values with 25th and 75th percentiles. For comparisons of the Δ leukocyte, Δ albumin, and Δ lysozyme levels, as well as of the Δ symptom scores at each time point among the challenges with 1, 10, and 100 μ g of capsaicin, Friedman's analysis of variance followed by the Wilcoxon signed-rank test were performed. Also, the fold increases from prechallenge levels of albumin and lysozyme were calculated, and comparison among the three capsaicin doses was performed with the same approach. For analysis of possible relationship(s) between the clinical symptoms and the biologic changes after capsaicin challenge, the Spearman rank correlation coefficient was determined. The average of Δ symptom scores at 10 seconds, 5 minutes, 10 minutes, and 30 minutes was calculated. This average of the acute Δ symptom scores and the

Δ symptom scores at 1 hour and 4 hours was correlated with the Δ leukocyte, Δ albumin, and Δ lysozyme at 30 minutes, 1 hour, and 4 hours by using pooled data from the three capsaicin dose protocols. A *p* value of less than 0.05 was considered significant.

RESULTS

Symptoms

Capsaicin nasal challenge induced symptoms of burning, rhinorrhea, and nasal congestion that developed acutely and faded over time. For burning, significant increases over prechallenge symptom scores were observed from 10 seconds to 10 minutes after 1 μ g of capsaicin and from 10 seconds to 1 hour after 10 μ g and 100 μ g of capsaicin (Fig. 2). For congestion, there was no significant increase in symptom score after 1 μ g of capsaicin; but significant increases were observed from 10 seconds to 10 minutes after 10 μ g of capsaicin and from 10 seconds to 4 hours after 100 μ g of capsaicin (Fig. 3). For rhinorrhea, significant increases over prechallenge symptom scores were observed from 10 seconds to 10 minutes, 30 minutes, and 4 hours after 1 μ g, 10 μ g, and 100 μ g of capsaicin, respectively (Fig. 4). Comparisons of changes from prechallenge levels (Δ symptom score) in burning, congestion, and rhinorrhea after the three capsaicin dosages show that at most time points these responses are dose-dependent (Friedman's ANOVA comparing 1 μ g, 10 μ g, and 100 μ g of capsaicin: *p* $\leq$ 0.008 from 10 seconds to 1 hour for burning; *p* $\leq$ 0.002 from 10 seconds to 30 minutes for congestion; and *p* $\leq$ 0.01 from 5 minutes to 30 minutes for rhinorrhea (Figs. 2 to 4).

Total leukocyte count

The total leukocyte content of nasal lavage fluids was not significantly increased from prechallenge levels after 1 μ g of capsaicin (Wilcoxon pre- vs postchallenge of 1

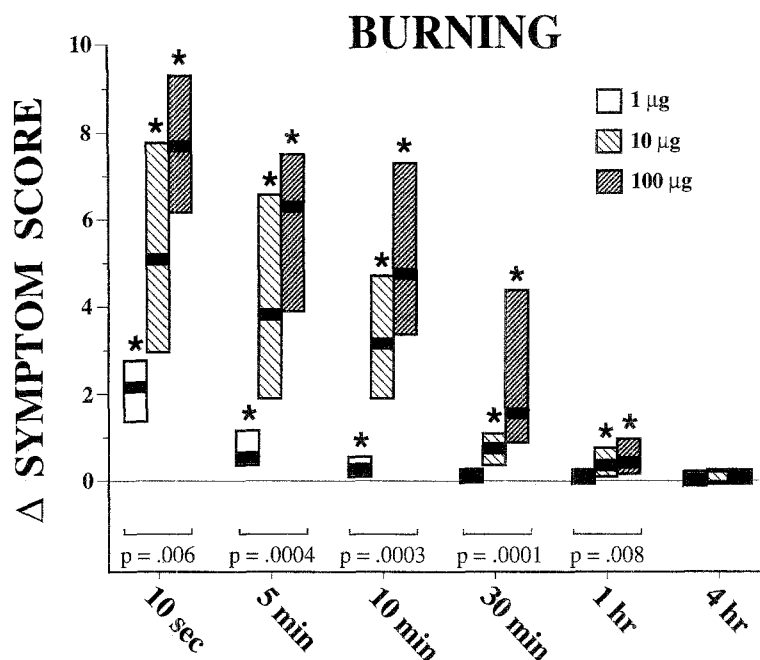


FIG. 2. Changes in symptom scores for burning from pre- to postchallenge with 1 µg, 10 µg, and 100 µg of capsaicin. Data are shown with modified box plots. Heavy marks within bars represent median values; top and bottom of bars represent 75th and 25th percentiles, respectively. Comparison of pre- and postchallenge symptom scores by Wilcoxon paired test showed significant increases ($p < 0.05$) in burning sensation at various time points after capsaicin administration. Comparison of Δ symptom scores within the three capsaicin doses by Friedman's ANOVA demonstrated significant differences at various time points as shown, suggesting that symptoms induced by capsaicin are mostly dose-dependent.

µg capsaicin: $p = 0.88$ at 30 minutes, $p = 0.96$ at 1 hour and $p = 0.09$ at 4 hours). On the other hand, there were significant increases in total leukocyte counts 1 hour and 4 hours after 10 µg of capsaicin (Wilcoxon pre- vs postchallenge of 10 µg of capsaicin: $p = 0.24$ at 30 minutes, $p < 0.05$ at 1 hour, and $p = 0.008$ at 4 hours); and 30 minutes, 1 hour, and 4 hours after 100 µg of capsaicin (Wilcoxon pre- vs postchallenge 100 µg of capsaicin: $p = 0.009$ at 30 minutes, $p = 0.007$ at 1 hour, and $p = 0.007$ at 4 hours). Comparison of changes from prechallenge leukocyte counts (Δ leukocytes) after the three capsaicin dosages indicates increasing cell influx with increasing capsaicin doses. At the 4 hour time point, a plateau is noted between the 10 and 100 µg doses (Wilcoxon Δ leukocytes 10 µg vs 100 µg of capsaicin: $p = 0.09$) (Fig. 5).

Differential cell counts

Analysis of the differential cell counts was performed within each capsaicin dose protocol to assess for a time effect of capsaicin challenge on the influx of various types of leukocytes. For this analysis we used the differences in absolute cell numbers between each time point and the prechallenge values. This was an acceptable approach because, within each capsaicin dose protocol, no significant difference in total leukocyte numbers was observed among the three time points (Friedman's ANOVA 30 minutes vs 1 hour vs 4 hours: $p = 0.12$ for 1 µg, $p = 0.07$ for 10 µg, and $p = 0.79$ for 100 µg).

Mononuclear cells, eosinophils, and neutrophils were not significantly increased at any time point after 1 µg of capsaicin (Table I). Neutrophil influx manifested the same pattern seen in the evaluation of total leukocyte counts, with significant increases 1 hour and 4 hours after 10 µg of capsaicin; and 30 minutes, 1 hour, and 4 hours after 100 µg of capsaicin. Mononuclear cell counts were not significantly different from prechallenge values at the 30-minute time point after any capsaicin dose but were significantly increased 4 hours after 10 µg of capsaicin and 1 hour and 4 hours after 100 µg of capsaicin. Eosinophils, on the other hand, were significantly increased 4 hours after 10 µg of capsaicin and 30 minutes after 100 µg of capsaicin.

We also analyzed the capsaicin dose effect on differential cell recruitment at each time point. In this analysis, percentage values were used because, at each time point, the three capsaicin dose protocols differed in terms of total leukocyte numbers (Fig. 5). Our analysis showed no significant difference in the percentage of mononuclear cells, eosinophils, or neutrophils among the three capsaicin doses, although there appeared to be more eosinophils with higher capsaicin doses at the 1-hour time point (Friedman's ANOVA 1 µg vs 10 µg vs 100 µg: $p = 0.05$).

Albumin

There was no significant increase in the albumin content of nasal lavage fluids at any time point after 1 µg

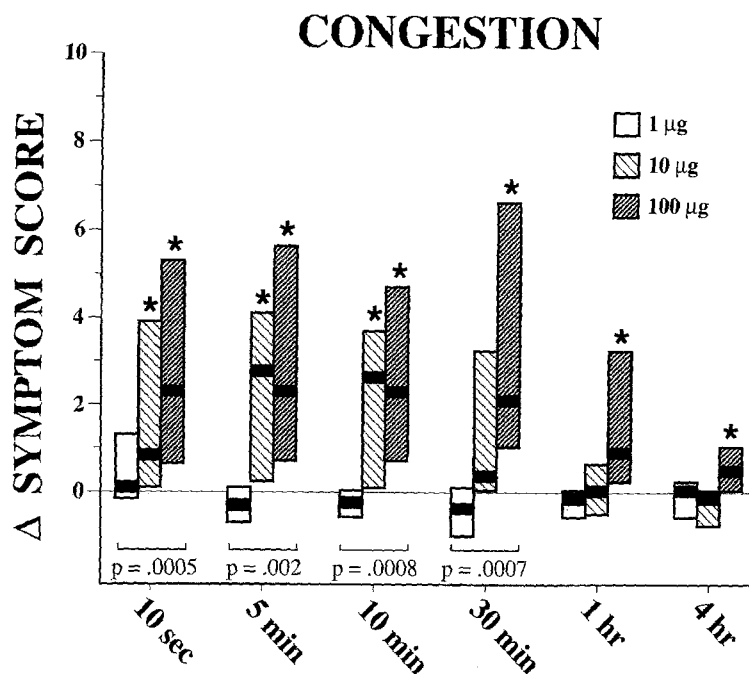


FIG. 3. Changes in symptom scores for congestion from pre- to postchallenge with 1 μ g, 10 μ g, and 100 μ g of capsaicin. Data are shown with modified box plots. Comparison of pre- and postchallenge symptom scores by Wilcoxon paired test showed significant increases (* $p < 0.05$) in nasal congestion at various time points after capsaicin administration. Comparison of Δ symptom scores within the three capsaicin doses by Friedman's ANOVA demonstrated significant differences at various time points as shown, suggesting that symptoms induced by capsaicin are mostly dose-dependent.

of capsaicin (Wilcoxon pre- vs postchallenge 1 μ g of capsaicin: $p = 0.33$ at 30 minutes, $p = 0.21$ at 1 hour, and $p = 0.77$ at 4 hours). On the other hand, there was a significant albumin increase 30 minutes after 10 μ g and 100 μ g capsaicin challenges (Wilcoxon pre- vs postchallenge 10 μ g of capsaicin: $p = 0.005$ at 30 minutes, $p = 0.96$ at 1 hour, and $p = 0.41$ at 4 hours; pre- vs postchallenge 100 μ g of capsaicin: $p = 0.005$ at 30 minutes, $p = 0.86$ at 1 hour, and $p = 0.58$ at 4 hours). Comparison of the changes from prechallenge albumin levels (Δ albumin) after the three capsaicin dosages at 30 minutes indicates that this is a dose-dependent response (Friedman's ANOVA: $p = 0.0007$) (Fig. 6). Comparison of fold increases in albumin over prechallenge levels among the three capsaicin dosages showed significant difference only at 30 minutes after challenge (Friedman's ANOVA, 1 μ g vs 10 μ g vs 100 μ g capsaicin: $p = 0.002$ at 30 minutes, $p = 0.58$ at 1 hour, and $p = 0.92$ at 4 hours after challenge) (Table II).

Lysozyme

There was no significant increase in lysozyme levels in the returned lavage fluids at any time point after 1 μ g of capsaicin (Wilcoxon pre- vs postchallenge 1 μ g of capsaicin: $p = 0.26$ at 30 minutes, $p = 0.68$ at 1 hour, and $p = 0.51$ at 4 hours). On the other hand, there was significant lysozyme increase 30 minutes after 10 μ g and 100 μ g capsaicin challenges (Wilcoxon pre- vs postchallenge 10 μ g of capsaicin: $p = 0.005$ at 30 minutes, $p =$

0.57 at 1 hour, and $p = 0.19$ at 4 hours; pre- vs postchallenge 100 μ g of capsaicin: $p = 0.005$ at 30 minutes, $p = 0.44$ at 1 hour, and $p = 0.20$ at 4 hours). Comparison of the changes from prechallenge lysozyme levels (Δ lysozyme) after the three capsaicin dosages at 30 minutes indicates that this is a dose-dependent response (Friedman's ANOVA: $p = 0.001$) (Fig. 7). Comparison of fold increases in lysozyme over prechallenge levels among the three capsaicin dosages showed significant difference only at 30 minutes after challenge (Friedman's ANOVA, 1 μ g vs 10 μ g vs 100 μ g of capsaicin: $p = 0.02$ at 30 minutes, $p = 0.60$ at 1 hour, and $p = 0.27$ at 4 hours after challenge). (Table II).

Relationships between symptoms and biologic responses

There were significant correlations between the acute Δ symptom scores for burning and the acute Δ lysozyme levels in nasal lavage fluids (Spearman rank correlation, average of Δ symptom scores for burning from 10 seconds to 30 minutes vs Δ lysozyme at 30 minutes: $r = 0.63$ and $p = 0.0007$); between the acute Δ symptom scores for rhinorrhea and the acute Δ lysozyme levels (Spearman rank correlation, average of Δ symptom scores for rhinorrhea from 10 seconds to 30 minutes vs Δ lysozyme at 30 minutes: $r = 0.52$ and $p = 0.005$); and between the acute Δ symptom scores for burning and the acute Δ albumin levels (Spearman rank correlation, average of Δ symptom scores for burning from 10

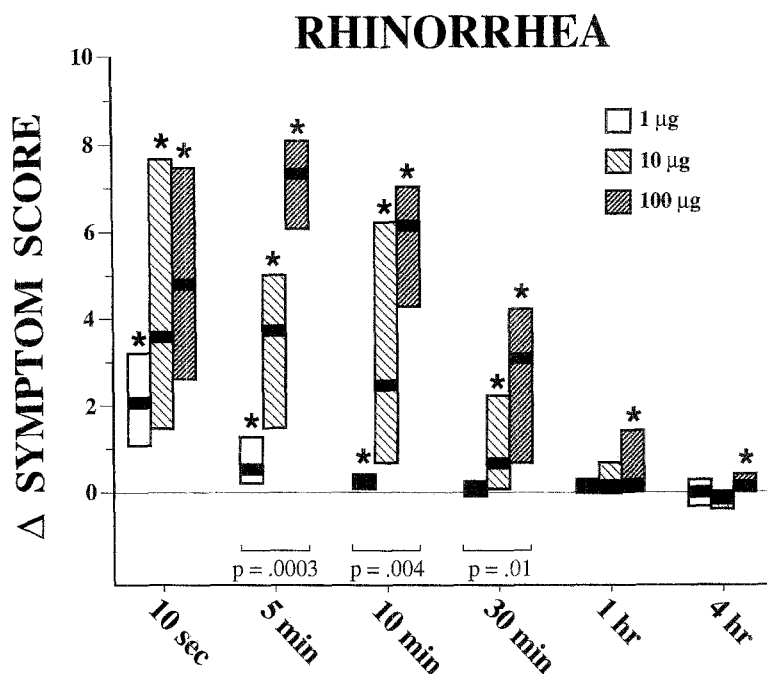


FIG. 4. Changes in symptom scores for rhinorrhea from pre- to postchallenge with 1 μ g, 10 μ g, and 100 μ g of capsaicin. Data are shown with modified box plots. Comparison of pre- and postchallenge symptom scores by Wilcoxon paired test showed significant increases (* $p < 0.05$) in rhinorrhea at various time points after capsaicin administration. Comparison of Δ symptom scores within the three capsaicin doses by Friedman's ANOVA demonstrated significant differences at various time points as shown, suggesting that symptoms induced by capsaicin are mostly dose-dependent.

seconds to 30 minutes vs Δ albumin at 30 minutes: $r = 0.53$ and $p = 0.004$).

DISCUSSION

Neuronal involvement in the pathophysiology of inflammatory respiratory disease has gained attention in recent decades.²⁷ In addition to adrenergic and cholinergic nerves, peptidergic nerve fibers have been identified in the human upper²⁸ and lower airways.²⁹ The role of peptidergic nerves in the development of airway inflammation has been demonstrated in many animal studies. In these models stimulation of these tachykinin-containing nerves produces leukocyte activation and plasma extravasation.³⁻⁷ The occurrence of such neurogenic inflammation in the human airway, however, has been largely unestablished.

We have recently demonstrated that capsaicin nasal spray produces significant increases in the leukocyte content of nasal lavage fluids at various time points after challenge.²² In the present study we found that this inflammatory cell influx is dose-dependent. Capsaicin at a 1 μ g dose did not produce any significant increase in the leukocyte counts of nasal lavage fluids at any time point. On the other hand, the 10 μ g dose produced significant leukocyte increases at two time points, whereas the 100 μ g dose produced significant increases at all three time points. This finding not only reproduces previous results reported by our group and others^{22, 23} in

terms of capsaicin-induced leukocyte influx but also strengthens its validity by demonstrating dose-response characteristics. The evidence of dose-dependent leukocyte recruitment in combination with dose-dependent albumin extravasation after sensory nerve stimulation confirms that neurogenic inflammation, as it has been previously described in animal models, does occur in the human airway.

There is abundant evidence suggesting that the capsaicin-induced inflammatory cell recruitment is mediated by tachykinins, particularly substance P. Capsaicin has been shown to induce significant release of substance P-like immunoreactivity in isolated guinea pig airways,^{30, 31} which is dose-dependent.³² Moreover, substance P has been demonstrated by multiple in vitro studies to induce leukocyte activation and chemotaxis in rats^{33, 34} and human beings.³⁵⁻³⁷ Braunstein et al.^{38, 39} have shown that in vivo nasal challenge with tachykinins in subjects with allergic rhinitis induces inflammatory cell recruitment in parallel with our present findings. To our knowledge, however, there has been no study directly demonstrating the release of substance P or other neuropeptides after capsaicin nasal challenge in human beings. This aspect needs further investigation. The occurrence of this event is suggested by the depletion of calcitonin gene-related peptide in the nasal mucosa of subjects with rhinitis after repeated application of capsaicin.⁴⁰

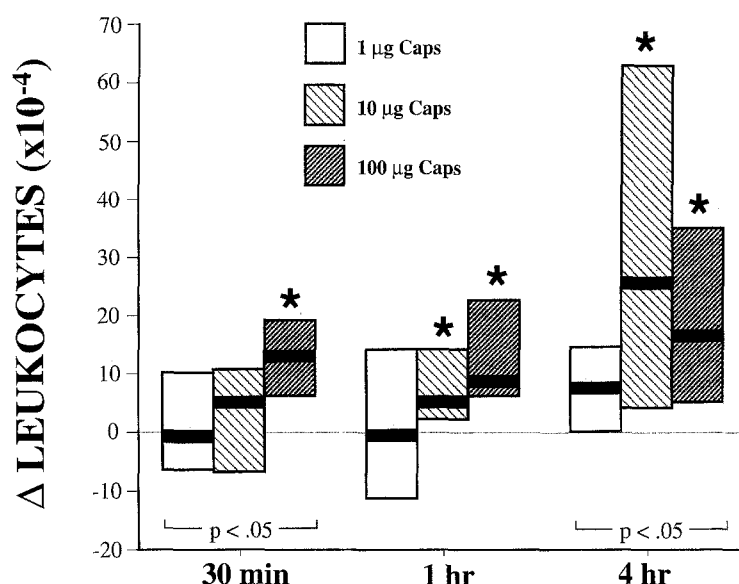


FIG. 5. Changes in leukocyte counts from before to after challenge with 1 μ g, 10 μ g, and 100 μ g of capsaicin. Comparison of pre- and postchallenge leukocyte counts by Wilcoxon paired test showed significant increases (* $p < 0.05$) 1 hour and 4 hours after 10 μ g of capsaicin. There was no significant leukocyte increase after 1 μ g of capsaicin. Comparison of change in leukocytes within the three capsaicin doses by Friedman's ANOVA indicated a trend toward greater leukocyte influx with increasing capsaicin doses, which plateaus at the 4-hour time point. Caps, Capsaicin.

TABLE I. Results of Wilcoxon signed-rank tests comparing total mononuclear cells, eosinophils, and neutrophils in nasal lavage fluids collected before and after challenge with 1 μ g, 10 μ g, and 100 μ g of capsaicin

	Capsaicin dose		
	1 μ g	10 μ g	100 μ g
Mononuclear cells			
Before vs after 30 min	$p > 0.999$	$p = 0.401$	$p = 0.249$
Before vs after 1 hr	$p = 0.612$	$p = 0.069$	$p = 0.028$
Before vs after 4 hr	$p = 0.515$	$p = 0.015$	$p = 0.028$
Eosinophils			
Before vs after 30 min	$p = 0.237$	$p = 0.484$	$p = 0.046$
Before vs after 1 hr	$p = 0.075$	$p = 0.401$	$p = 0.173$
Before vs after 4 hr	$p = 0.263$	$p = 0.036$	$p = 0.075$
Neutrophils			
Before vs after 30 min	$p = 0.779$	$p = 0.327$	$p = 0.046$
Before vs after 1 hr	$p = 0.499$	$p = 0.038$	$p = 0.028$
Before vs after 4 hr	$p = 0.139$	$p = 0.008$	$p = 0.028$

The dose dependency of leukocyte influx after capsaicin nasal challenge correlates well with the study results of Umeno et al.³ They demonstrated that capsaicin administration in the rat trachea results in immediate adherence of neutrophils to vascular endothelium with relatively little migration out of the vessels. However, with the effective increase in stimulus through the inhi-

bition of neuropeptide degradation, they found that cell transmigration was increased.

Albumin leakage after capsaicin-sensitive nerve stimulation in the human nose is likewise a dose-dependent phenomenon. In this study we noted increasing albumin levels in nasal lavage fluids immediately after capsaicin administration at doses of 10 μ g and 100 μ g. There was no significant increase in albumin after the application of capsaicin at 1 μ g. These findings may explain the lack of capsaicin-induced albumin leakage in previous studies,^{21, 41} in which the dose delivered was less than 10 μ g. Our results correspond with the findings of Asakura et al.⁷ in guinea pigs, in which dose-dependent leakage of intravenous dye was induced by topical capsaicin nasal challenge. The increased vascular permeability after capsaicin application may again be mediated by released tachykinins. Braunstein et al.³⁸ have demonstrated that tachykinin nasal challenge in subjects with allergic rhinitis produces increased albumin levels in nasal lavage fluids.

Our use of relatively higher capsaicin doses compared with those used in previous investigations may suggest that the observed plasma leakage could be due to a nonspecific, even a toxic, effect and not to neuronal activation. However, albumin increase in this study only occurred at the initial time point after capsaicin challenge and not thereafter. The absence of continuous plasma extravasation after this acute response thus reduces the possibility of direct and longer-lasting disruption of vascular integrity.

We have previously shown that capsaicin nasal challenge produces increased glandular secretion.¹⁹ The

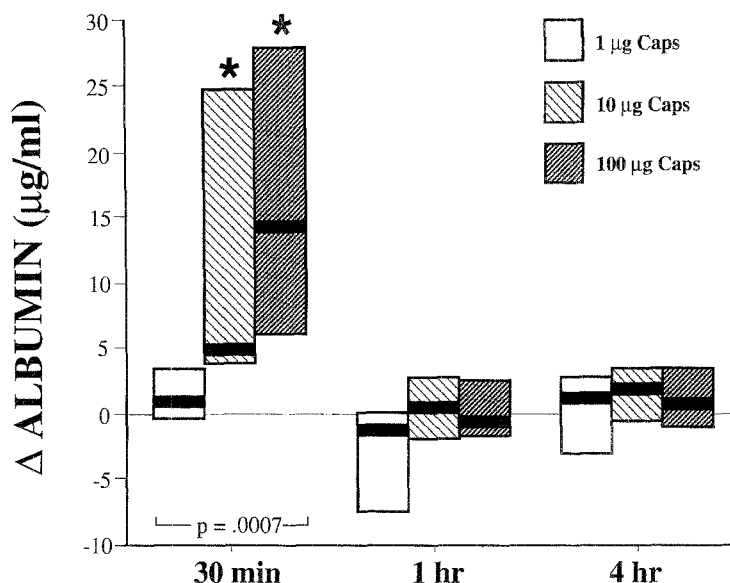


FIG. 6. Changes in albumin levels from before to after challenge with 1 μ g, 10 μ g, and 100 μ g of capsaicin. Comparison of pre- and postchallenge albumin levels by Wilcoxon paired test showed significant increases (* $p < 0.05$) after 10 μ g and 100 μ g of capsaicin at the 30-minute time point. There was no significant albumin increase after 1 μ g of capsaicin at any time point. Comparison of Δ albumin within the three capsaicin doses by Friedman's ANOVA indicated that acute albumin leakage is dose-dependent ($p = 0.0007$ at 30 minutes). Caps, Capsaicin.

present study demonstrates that this response is also dose-dependent, as manifested by the increasing levels of lysozyme after administration of increasing doses of capsaicin. This result correlates with findings in animal models, in which capsaicin induces dose-dependent increases in nasal secretions.¹¹ Of interest, the acute increase in lysozyme levels in the nasal lavage fluids after capsaicin challenge correlated with the acute symptoms of burning and rhinorrhea. Furthermore, the acute symptom of burning also correlated with the capsaicin-induced acute increase in the albumin levels. This supports the concept that both the acute symptoms and the biologic changes observed in nasal lavage fluids are manifestations of the same physiologic phenomenon.

The fold increases in the lysozyme content of nasal lavage fluids after capsaicin challenge were larger than those of albumin. This result is not surprising, if we accept the assumption that capsaicin activates submucosal gland secretion through two additive mechanisms: (1) through an axonal reflex with local antidromic release of neuropeptides and (2) through a central neuronal reflex with a parasympathetic efferent arm. In contrast, vascular permeability may be solely the result of local antidromic release of neuropeptides.

As in the case of albumin, the increase in lysozyme was only evident at the 30-minute time point after capsaicin challenge. The increase in total leukocytes, on the other hand, was observed up to 4 hours after challenge. These differences in the temporal pattern of the vascular-glandular versus leukocytic responses indicate that acute and late responses to sensory nerve stimulation, reminiscent of the events after allergen

TABLE II. Fold increases over prechallenge levels of albumin and lysozyme

	Capsaicin dose		
	1 μ g	10 μ g	100 μ g
Albumin			
30 min	1.2 (0.9, 1.5)	2.7 (1.5, 4.5)	4.6 (2.0, 8.9)
1 hr	0.7 (0.4, 1.0)	1.2 (0.5, 1.5)	1.0 (0.6, 2.2)
4 hr	1.2 (0.5, 1.8)	1.2 (0.8, 2.2)	1.3 (0.6, 2.1)
Lysozyme			
30 min	1.9 (1.1, 2.8)	3.0 (1.7, 27.8)	16.1 (11.3, 36.9)
1 hr	1.2 (0.7, 1.6)	1.0 (0.7, 2.4)	1.5 (0.7, 3.2)
4 hr	1.6 (0.6, 2.6)	1.2 (1.0, 1.7)	2.6 (0.8, 4.3)

Data are expressed as medians with 25th, 75th percentiles in parentheses.

challenge, may exist. The differences in the acute and late responses to capsaicin application may suggest two separate phases of neurogenic inflammation or even two separate inflammatory mechanisms. It is interesting to note that significant increases in mononuclear cell counts were observed only during the later time points and not during the initial collection of lavage samples. Recruitment of eosinophils and neutrophils, on the other hand, was noted at both the early and later time points after capsaicin nasal challenge.

That these nasal responses to capsaicin challenge were evident in subjects with active allergic rhinitis suggests that having a concomitant allergic condition may enhance responsiveness to neuronal stimulation. Indeed, animal studies have demonstrated that allergen expo-

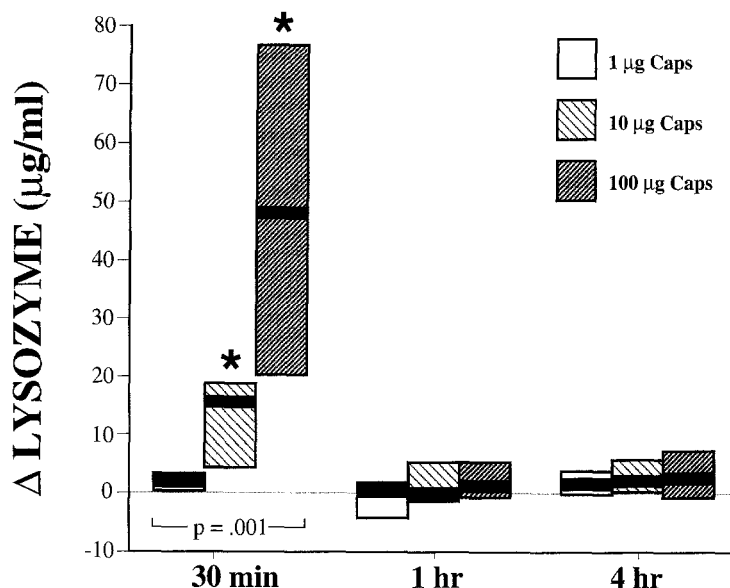


FIG. 7. Changes in lysozyme levels from before to after challenge with 1 μ g, 10 μ g, and 100 μ g of capsaicin. Comparison of pre- and postchallenge lysozyme levels by Wilcoxon paired test showed significant increases ($*p < 0.05$) after 10 μ g and 100 μ g of capsaicin at the 30-minute time point. There was no significant increase in lysozyme after 1 μ g of capsaicin at any time point. Comparison of Δ lysozyme within the three capsaicin doses by Friedman's ANOVA indicated that acute increase in this glandular product is dose-dependent ($p = 0.001$ at 30 minutes). Caps, Capsaicin.

sure increases the effects of nerve stimulation in the airways.^{42, 43} This may be true in the case of plasma extravasation, because our earlier study has shown that capsaicin-induced albumin leakage occurs in subjects with allergic rhinitis but not in subjects with nonallergic rhinitis or in normal control subjects.²⁴ On the other hand, glandular hypersecretion after capsaicin application is comparable between subjects with allergic rhinitis and healthy control subjects.^{23, 24} Meanwhile, leukocyte influx after capsaicin nasal challenge in healthy subjects has been documented by our group²² but not by others.²³ Increased vascular permeability therefore appears to be the nasal inflammatory response to neuronal stimulation that is unique to those with active allergic rhinitis. Further studies are needed to elucidate the effective relationship between neuronal and allergen stimulation.

In conclusion, our study demonstrates that the capsaicin-induced nasal inflammatory cell recruitment, plasma extravasation, and glandular hypersecretion are reproducible and dose-dependent. These results provide in vivo evidence that neurogenic inflammation, a phenomenon that has been well demonstrated in various animal species, can also occur in the human upper airway with active allergic disease.

REFERENCES

- Jancso N, Jancso-Gabor A, Szolcsanyi J. Direct evidence for neurogenic inflammation and its prevention by denervation and by pre-treatment with capsaicin. *Br J Pharmacol Chemother* 1967;31:138-51.
- Pernow B. Role of tachykinins in neurogenic inflammation. *J Immunol* 1985;135:812-5.
- Umeno E, Nadel JA, McDonald DM. Neurogenic inflammation of the rat trachea: fate of neutrophils that adhere to venules. *J Appl Physiol* 1990;69:2131-6.
- McDonald DM. Neurogenic inflammation in the rat trachea. I. changes in venules, leucocytes and epithelial cells. *J Neurocytol* 1988;17:583-603.
- McDonald DM, Mitchell RA, Gabella G, Haskell A. Neurogenic inflammation in the rat trachea. II. Identity and distribution of nerves mediating the increase in vascular permeability. *J Neurocytol* 1988;17:606-28.
- Lundblad L, Saria A, Lundberg JM, Anggard A. Increased vascular permeability in rat nasal mucosa induced by substance P and stimulation of capsaicin-sensitive trigeminal neurons. *Acta Otolaryngol* 1983;96:479-84.
- Asakura K, Shirasaki H, Narita S, Kojima T, Kataura A. Study on the dye leakage response of nasal mucosa following topical, capsaicin challenge in guinea pigs. *Acta Otolaryngol* 1992;112:545-51.
- Tanaka DT, Grunstein MM. Mechanisms of substance P-induced contraction of rabbit airway smooth muscle. *J Appl Physiol* 1984;57:1551-7.
- Sekizawa K, Tamaoki J, Nadel JA, Borson DB. Enkephalinase inhibitor potentiates substance P- and electrically induced contraction in ferret trachea. *J Appl Physiol* 1987;63:1401-5.
- Asakura K, Narita S, Kojima T, Saito H, Kataura A. Changes in nasal airway resistance and secretory response in the guinea pig after nasal challenge with capsaicin and histamine. *Eur Arch Otorhinolaryngol* 1994;251:224-8.
- Mizoguchi H, Bergeron ML. Effect of capsaicin on nasal secretion in anesthetized ferrets. *J Appl Physiol* 1991;70:282-6.
- Djukanovic R, Roche WR, Wilson JW, Beasley CR, Twentyman OP, Howarth PH, et al. Mucosal inflammation in asthma. *Am Rev Respir Dis* 1990;142:434-57.
- Knani J, Campbell A, Enander I, Peterson CG, Michel FB, Bousquet J. Indirect evidence of nasal inflammation assessed by titration of inflammatory mediators and enumeration of cells in nasal secre-

- tions of patients with chronic rhinitis. *J Allergy Clin Immunol* 1992;90:880-9.
14. Barnes PJ. Neuropeptides and asthma. *Am Rev Respir Dis* 1991;143:S28-S32.
15. Barnes PJ. Neurogenic inflammation in airways. *Int Arch Allergy Appl Immunol* 1991;94:303-9.
16. Togias A, Naclerio RM, Proud D, Baumgarten CR, Peters SP, Warner J, et al. Mediator release during nasal provocation: a model to investigate the pathophysiology of rhinitis. *Am J Med* 1986;79(suppl 6A):26-33.
17. Persson CGA, Svensson C, Greiff L, Andersson M, Wollmer P. The use of the nose to study the inflammatory response of the respiratory tract [editorial]. *Thorax* 1992;47:993-1000.
18. Holzer P. Capsaicin: cellular targets, mechanisms of action, and selectivity for thin sensory neurons. *Pharmacol Rev* 1991;43:143-201.
19. Philip G, Baroody FM, Proud D, Naclerio RM, Togias AG. The human nasal response to capsaicin. *J Allergy Clin Immunol* 1994;94:1035-45.
20. Geppetti P, Fusco BM, Marabini S, Maggi CA, Fanciullacci M, Sicuteri F. Secretion, pain and sneezing induced by the application of capsaicin to the nasal mucosa in man. *Br J Pharmacol* 1988;93:509-14.
21. Bascom R, Kagey-Sobotka A, Proud D. Effect of intranasal capsaicin on symptoms and mediator release. *J Pharmacol Exp Ther* 1991;259:1323-7.
22. Philip G, Sanico AM, Togias A. Inflammatory cellular influx follows capsaicin nasal challenge. *Am J Respir Crit Care Med* 1996;153:1222-9.
23. Roche N, Lurie S, Authier S, Dusser DJ. Nasal response to capsaicin in patients with allergic rhinitis and in healthy volunteers: effect of colchicine. *Am J Respir Crit Care Med* 1995;151:1151-8.
24. Philip G, Proud D, Naclerio RM, Togias AG. Increased responses to capsaicin nasal challenge in allergic versus nonallergic rhinitis [abstract]. *J Allergy Clin Immunol* 1994;93:217.
25. Naclerio RM, Meier HL, Kagey-Sobotka A, Adkinson NF Jr, Meyers DA, Norman PS, et al. Mediator release after nasal airway challenge with allergen. *Am Rev Respir Dis* 1983;128:597-602.
26. Riccio MM, Reynolds CJ, Hay DW, Proud D. Effects of intranasal administration of endothelin-1 to allergic and nonallergic individuals. *Am J Respir Crit Care Med* 1995;152:1757-64.
27. Barnes PJ. Asthma as an axon reflex. *Lancet* 1986;1:242-5.
28. Baraniuk JN, Lundgren JD, Okayama M, Goff J, Mullol J, Merida M, et al. Substance P and neurokinin A in human nasal mucosa. *Am J Respir Cell Mol Biol* 1991;4:228-36.
29. Lundberg JM, Hokfelt T, Martling CR, Saria A, Cuello C. Substance P-immunoreactive sensory nerves in the lower respiratory tract of various mammals including man. *Cell Tissue Res* 1984;235:251-61.
30. Saria A, Theodorsson-Norheim E, Gamse R, Lundberg JM. Release of substance P- and substance K-like immunoreactivities from the isolated perfused guinea-pig lung. *Eur J Pharmacol* 1985;106:207-8.
31. Saria A, Martling CR, Elvar ZV, Theodorsson-Norheim E, Gamse R, Lundberg JM. Release of multiple tachykinins from capsaicin-sensitive sensory nerves in the lung by bradykinin, histamine, dimethylphenyl piperazinium, and vagal nerve stimulation. *Am Rev Respir Dis* 1988;137:1330-5.
32. Martins MA, Shore SA, Drazen JM. Capsaicin-induced release of tachykinins: effects of enzyme inhibitors. *J Appl Physiol* 1991;70:1950-6.
33. Helme RD, Eglezos A, Hosking CS. Substance P induces chemotaxis of neutrophils in normal and capsaicin-treated rats. *Immunol Cell Biol* 1987;65:267-9.
34. Roch-Arveiller M, Regoli D, Chanaud B, Lenoir M, Muntaner O, Stralzo S, et al. Tachykinins: effects on motility and metabolism of rat polymorphonuclear cells. *Pharmacology* 1986;33:266-73.
35. Wiedermann CJ, Wiedermann FJ, Apperl A, Kieselbach G, Gonalwinka G, Braunsteiner H. In vitro human polymorphonuclear leukocyte chemokinesis and human monocyte chemotaxis are different activities of aminoterminal and carboxyterminal substance P. *Naunyn Schmiedeberg Arch Pharmacol* 1989;340:185-90.
36. Kolasinski SL, Haines KA, Siegel EL, Cronstein BN, Abramson SB. Neuropeptides and inflammation: a somatostatin analog as a selective antagonist of neutrophil activation by substance P. *Arthritis Rheum* 1992;35:369-75.
37. Carolan EJ, Casale TB. Effects of neuropeptides on neutrophil migration through noncellular and endothelial barriers. *J Allergy Clin Immunol* 1993;92:589-98.
38. Braunstein G, Fajac I, Lacronique J, Frossard N. Clinical and inflammatory responses to exogenous tachykinins in allergic rhinitis. *Am Rev Respir Dis* 1991;144:630-5.
39. Braunstein G, Buvry A, Lacronique J, Desjardins N, Frossard N. Do nasal mast cells release histamine on stimulation with substance P in allergic rhinitis? *Clin Exp Allergy* 1994;24:922-9.
40. Lacroix JS, Buvelot JM, Polla BS, Lundberg JM. Improvement of symptoms of non-allergic chronic rhinitis by local treatment with capsaicin. *Clin Exp Allergy* 1991;21:595-600.
41. Greiff L, Svensson C, Andersson M, Persson CG. Effects of topical capsaicin in seasonal allergic rhinitis. *Thorax* 1995;50:225-9.
42. Ballati L, Evangelista S, Manzini S. Repeated antigen challenge induced airway hyperresponsiveness to neurokinin A and vagal non-adrenergic, non-cholinergic (NANC) stimulation in guinea pigs. *Life Sci* 1992;51:119-24.
43. Ellis JL, Udem BJ. Antigen-induced enhancement of noncholinergic contractile responses to vagus nerve and electrical field stimulation in guinea pig isolated trachea. *J Pharmacol Exp Ther* 1992;262:646-53.